



## **Serum Selenium, Zinc, and Copper in Early Diagnosed Patients with Pemphigus Vulgaris**

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### **Abstract**

**Background:** Pemphigus vulgaris is a life threatening, blistering skin disease. It is an autoimmune abnormality. Due to involvement of oral cavity and pharynx, patients are at risk of nutrients deficiency. The aim of this study was to evaluate the status of selenium, copper, and zinc in these patients.

**Methods:** In a case-control study, 43 newly diagnosed pemphigus vulgaris patients were compared with 58 healthy people from 2009 to 2010. The severity of the disease was estimated according to Harman's scores. Serum selenium was measured with atomic absorption but serum zinc and copper concentrations were determined spectrophotometrically. Data were compared with independent *t* test. Correlations were evaluated by Pearson correlation test.

**Results:** Both groups were the same based on sex, age, and weight and body mass index. The mean duration of disease was 5.6 month. The oral and skin severities were 1.79 and 2.3 respectively, based on Harman's scores. Serum selenium of pemphigus patients was significantly less than that of healthy people ( $P<0.001$ ). Serum copper was negatively correlated with duration of disease in males ( $P=0.02$ ,  $r=-0.5$ ).

**Conclusions:** Pemphigus vulgaris negatively affects on serum selenium, copper and zinc. It seems that serum selenium, copper and zinc decrease as the disease lasts longer.

**Keywords:** Selenium, Zinc, Copper, Pemphigus vulgaris

### **Introduction**

Pemphigus as a word originates from pemphix, a Greek word that means blister. Pemphigus is a life threatening skin disease (1). It possesses two subtypes, vulgaris, and foliaceus. Pemphigus vulgaris is the most common form of pemphigus in many territories. It is especially common among Jews and Mediterranean peoples. Nearly 91.9% of all cases with pemphigus belong to pemphigus vulgaris (2). The incidence of pemphigus vulgaris var-

ies from 0.07 to 3.2 cases per 100000 people per year, but it depends on geographical location and is nearly 1 per 100000 population in Iran (2). The mean age in which the disease starts is 42 years in Tehran (3). Pemphigus vulgaris affects females a bit more than males, therefore male to female ratio is 1 to 1.5 (3). Its mortality rate is 5% to 25% depending on the population under study (4). It is an autoimmune disorder, which causes painful

bullae in skin and mucus membranes (3). It is mediated by autoantibodies, which are directed to desmosomes, adhesion molecule between keratinocytes. This process leads to acantholysis and epidermal blisters formation. Genetic predilection is seemed to participate in pemphigus etiology. The genetic factors of pemphigus extend from MHC complex to the desmoglein 3 genes (5). Besides the genetics, environmental factors influence the disease incidence. These environmental factors could include drugs, chemicals, foods, physical agents, and stress.

Selenium as a trace element is a cofactor for antioxidant enzymes. Therefore, it is essential in antioxidant defense against free radicals. Copper also is crucial for tissue repair, due to its role in enzymes catalyzing cross bond in collagen and elastines. Zinc is necessary for anabolic pathways and its deficiency has negative effect on wound repair. Chronic inflammation causes zinc deficiency (6, 7). Zinc plays an anti-inflammatory and antioxidant role. It also increases immunity against pathogens (8, 9).

Mucosal involvement is an independent prognostic factor for poor prognosis in pemphigus (4). The mucosal involvement affects negatively on prognosis. It seems that mucosal ulcers disturb nutrient intakes. Therefore, we expect nutrient deficiency in pemphigus vulgaris. In this way, this study was designed to evaluate that hypothesis.

## Materials and Methods

This study was a case control research on 43 patients with pemphigus vulgaris, 16 to 69 years old, and 58 healthy persons, 22 to 60 years old. The patients were selected among ones referred to dermatology clinic in a teaching hospital. Pemphigus vulgaris was diagnosed based on clinical symptoms and immunology and histology findings. The inclusion criteria for patients were taking no supplements, not under treatment for pemphigus, not receiving corticosteroids. Women taking contraceptives were excluded. Patients with any hepatic or renal disorders were not enrolled.

Healthy people were recruited from volunteers. They should have had no history of autoimmune

diseases or inflammatory disorders. Ones taking supplements, corticosteroids and contraceptives were not enrolled. They were not affected with hypertension, cardiovascular diseases, and diabetes. The severity of disease was measured based on Harman's scores (10). It was evaluated by a single physician avoiding inter-individual variations due to observers. The extent of involvements of skin and mucus was recorded separately.

The patients and controls were interviewed about past medical history, drugs, and age. We measured their body weight to the nearest 0.1 kg with minimal clothing and without shoes, by a beam balance. Height was evaluated with a stadiometer to the nearest 0.5 cm without shoes and in upright position. Body mass index (BMI) was the product of weight (kg)/ height (m)<sup>2</sup> equation.

Blood sampling were done after 12 to 14 hours fasting from antecubital vein. Bloods were centrifuged at 3000 rpm for 10 min. The separated sera were immediately stored at -80°C until analysis.

Serum copper was indicated spectrophotometrically with RANDOX kit (Cat. No. CU 2340; Randox Labs Ltd., Crumlin, UK) according to instructions introduced by manufacturer. In this method, at pH 4.7 copper is released from ceruloplasmin in the presence of a reducing agent. Then, it reacts with a specific color reagent forming a stable and colored chelate. The intensity of the color is directly proportional to the amount of copper in the sample. Its concentration is expressed in µmol/l.

Serum zinc was measured with RANDOX kit (Cat. No. ZN 2341; Randox Labs Ltd., Crumlin, UK) according to instructions introduced by manufacturer. Zinc present in serum is chelated first and then the result complex is measured at a wavelength of 560 nm. Zinc concentration is also expressed in µmol/l.

Serum selenium was measured based on Jacobson's method (11). It was done by atomic absorption. Its concentration was expressed in µg/dl.

## Statistical analysis

All data are expressed in mean±standard error (SE). Normality of distribution was evaluated by Kolmogorov-Smirnov test. Groups were com-

pared with independent sample t test in the case of continues variable, but chi square test used for categorical data. Correlation was measured with Pearson's coefficient and Spearman's rho depending on the type of variables. A value of  $P < 0.05$  was considered to be statistically significant. Statistical analysis was carried out with the statistical Package for the Social Sciences (SPSS) (Version 11.5, SPSS Inc, Chicago, IL, USA).

## Results

Forty-three pemphigus vulgaris patients fulfilled the inclusion criteria and enrolled in the study. They consisted of 21 males and 22 females. The mean duration of disease was  $5.62 \pm 0.17$  months. The mean severity score of skin involvement was  $2.3 \pm 0.15$ , and  $1.79 \pm 0.17$  for oral involvement. Control group was consisted of 58 healthy persons including 22 males and 36 females. Both groups were statistically the same based on sex distribution, age, weight, height, and BMI (Table 1).

**Table 1:** Sex distribution, anthropometric indexes and age of patients and healthy persons\*

	Patients (43)	Controls (58)	P value
Sex (male/female)	21/22	22/36	0.27 <sup>1</sup>
Age (year)	$42.3 \pm 1.76$	$43.7 \pm 1.36$	0.5 <sup>2</sup>
Weight (kg)	$65.97 \pm 2.15$	$67.05 \pm 1.64$	0.68 <sup>2</sup>
Height (cm)	$163.58 \pm 1.24$	$161.67 \pm 1.08$	0.25 <sup>2</sup>
BMI (kg/m <sup>2</sup> )	$24.6 \pm 0.76$	$25.75 \pm 3.87$	0.22 <sup>2</sup>

\* Data are means±S.E.

1- Chi square test

2- Independent sample t test

The concentrations of serum zinc, selenium, and copper in patients and healthy subjects were shown in Table 2. The serum zinc was higher in healthy subjects, but it was not statistically significant. Serum selenium also was less in patients and it was significant statistically ( $P < 0.001$ ).

**Table 2:** Serum concentrations of zinc, selenium and copper in patients and controls\*

	Patients (43)	Controls (58)	P value <sup>1</sup>
Serum zinc (µmol/l)	$13.85 \pm 0.89$	$15.11 \pm 0.51$	0.23
Serum selenium (µg/dl)	$80.34 \pm 1.55$	$92.85 \pm 1.73$	<0.001
Serum copper (µmol/l)	$12.95 \pm 0.97$	$14.39 \pm 0.62$	0.21

\* Data are means±S.E.

1- Independent sample t test

The correlations of three trace elements with duration of disease were negative, but they were insignificant statistically. We found a positive correlation between serum zinc and copper ( $r=0.52$ ,  $P < 0.001$ ). The correlations of serum zinc, selenium and copper with duration and severity of disease are depicted in Table 3.

**Table 3:** Correlations of serum zinc, copper and selenium with duration of disease and the severity

		Duration <sup>1</sup>	Skin severity <sup>2</sup>	Oral severity <sup>2</sup>
Serum zinc	<i>r</i>	-0.045	0.024	-0.104
	<i>P</i> value	0.77	0.87	0.50
Serum selenium	<i>r</i>	-0.071	0.045	0.12
	<i>P</i> value	0.65	0.77	0.43
Serum copper	<i>r</i>	-0.073	0.22	-0.129
	<i>P</i> value	0.64	0.145	0.41

1- Pearson's correlation coefficient

2- Spearman's rho

As it is shown, the correlations are not significant, but a negative and significant correlation was found between serum copper and duration of disease in males only ( $r=-0.5$ ,  $P=0.02$ ).

## Discussion

In this study, the results show that pemphigus vulgaris causes depletion of some trace elements including zinc, selenium and copper. It also indi-

cates that, as the duration of disease lasts more, the status of these trace elements would be got more aggravated. Although we could not find significant difference between groups for zinc and copper, the data show that pemphigus patients still have less serum copper and zinc. This finding might exist before the disease started. Even though we could not reject this hypothesis, but the correlations between serum elements and disease duration do not support this hypothesis. Instead, it shows that pemphigus vulgaris negatively affects zinc, copper and selenium statuses.

Negative correlations between disease duration and serum zinc, copper and selenium show that the patients lose more trace elements during disease or it may be due to inadequate intake. We could not find any association between severity and serum zinc, copper or selenium concentration. Because zinc and copper have beneficial roles in immune system, it seems that their deficiencies could increase morbidity and even mortality. Copper also have roles in wound healing. Selenium is an important component of antioxidant defense. Moreover under inflammatory conditions an oxidative stress occurs. Chronic inflammation could also cause zinc deficiency (7).

Balo-Banga and et al. measured copper, zinc, and manganese in the chromatin of peripheral mononuclear cells in pemphigus. Their findings support the results of this study but they did not check selenium status (12). Moreover, they studied only four patients and the patients were under corticosteroid therapy. Treatment could play as a confounding factor. Tasaki studied 151 patients with skin cancer, inflammatory and noninflammatory skin disease (13). They found that the patients had less serum zinc. They could not prove that about copper. Trace element depletion also could be the result of exudates from bulla. This association supported by Berger's study on burned patients (6). In burns serum copper, selenium and iron will decrease and the urinary depletion of selenium and zinc would increase. Moreover, supplementation with trace elements will decrease the infections rate (14-17).

In autoimmune diseases, serum selenium and zinc decrease but serum copper increases (18).

In conclusion, pemphigus vulgaris could cause copper, zinc and selenium deficiencies. It seems that their supplementations could alleviate the disease severity and mortality, but it needs some clinical trials to confirm.

## Ethical considerations

Patients and controls were deeply informed about the procedures and purposes of the study. They were free to leave the study. All of subjects signed written informed consent form before entering the study. In situation of being under 18 years old, one of parents signed written informed consent form. Research protocol was approved by Ethics Committee of Tehran University of Medical Sciences.

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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